The Search for Terrestrial Nanobacteria as Possible Analogs for Purported Martian Nanofossils in the Martian Meteorite ALH84001

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Introduction The recent report of possible nanofossils in the martian meteorite ALH84001[1] indicates that a greater emphasis on the study of terrestrial nanobacteria is necessary to determine if likely terrestrial analogs exist. Putative fossilized bacteria are reported from terrestrial rocks that are similar in age to the martian meteorite [2]; however, these fossils are considerably larger in size than those reported from ALH84001. Terrestrial nanobacteria have been observed in sedimentary rocks [*e.g.*,3,4]; these bacteria are within the same size range as those from the martian meteorite. In addition, nanobacteria have been reported for the first time from blood [5]. In order to examine igneous rock types similar to the main lithology of ALH84001, we are studying basalts from the Columbia River (CRB), which are 6-17 Ma old (Miocene). Although these basalts contain little organic carbon, populations of anaerobic microorganisms have been detected [6]. The CRB samples were obtained from several hundred meters below any sedimentary rocks and contained microorganisms that produced a variety of end products including methane [6]. Photosynthesis was not used as an energy source by these microorganisms; rather, it is suggested [6] that H_2 produced from water-basalt interaction via iron in the silicates within the basalt could serve as an energy source. Therefore, the CRB samples could be a close biochemical analog for the hypothesized ALH84001 ecosystem. We have examined CRB surfaces using high resolution scanning and transmission electron microscopy for microorganisms *in situ* and those extracted from the basalt surface.

Methods Four CRB sample surfaces were examined with a high resolution Philips XL 40 field emission gun scanning electron microscope (FEGSEM): DB-11, DC-06, P4 biomass, and reference basalt. The DB-11 and DC-06 were incubated for two years, in contact with anaerobic CRB groundwater and its entrained bacteria [technique described in 6]. DB-11 is a low-sulfate groundwater and DC-06 is a high sulfate ground water. P4 biomass had a large amount of biomass attached to the basalt and was placed in an anaerobic solution of mineral salts and inorganic nutrients to favor continued growth of bacteria [6]. The reference basalt was not exposed to water [6]. In addition to FEGSEM analysis of surfaces of CRB chips, some P4 biomass chips were treated with 2% glutaraldehyde for the present study (to fix any unfossilized bacteria by cross linking polysaccharides), rinsed with triple distilled water exposed to UV light source, placed in an ultrasonic bath for five minutes, and the residual liquid placed on copper grids with a formvar support film for analysis by transmission electron microscopy (TEM).

All four types of basalt samples were also analyzed for the presence of DNA using 4'-6'-diamidino-2-phenylindole (DAPI) by fluorescence microspectrophotometry [technique described in 7].

Results and Discussion Prior to this work, the presence of living, normal-sized (~ 1 μm in size) bacteria in the CRB was confirmed by measuring the rates of microbial activities in laboratory microcosms [6]. In addition, DNA analyses showed that the DC-11, DC-06, and P4 biomass samples contained normal-sized cells with at least one nucleoid of DNA. DC-06 and DB-11showed numerous bacteria, mostly rod-shaped. P4 biomass had clumps of up to 16 or more cells in each clump; nucleoids were present in P4 biomass. No bacteria was observed from the reference basalt.

FEGSEM observations show the presence of small, nanometer-scale coccoid (spheroidal) bacteria on DC-06 and DB-11. Intertwined tubular bodies (Fig. 1) were also found on DC-06, DB-11, and P4 biomass. The P4 biomass surface revealed clumps of these bodies up to ~2 x 1 μm in size. Individual tubes range from ~20-30 nm wide and up to several micrometers in length. They occur most commonly as masses of tubular bodies but are also found as individual filaments (Fig. 2). These tubular bodies are rich in Fe and contain lesser amounts of Ca, P, and Si. We suggest that these forms may be nanobacteria or appendages (e.g., filaments, fimbrae, or prothecae) of bacteria from the CRB samples. Another possibility is that they may be *dwarf* bacteria (< 0.3 µm in diameter); studies have shown that the predominant form of bacteria in subsurface environments is dwarf bacteria and it is unknown if these dwarf cells represent unique populations of small-sized bacteria or if they are different (starved) forms of commonly observed bacteria [e.g., 8]. Preliminary results from TEM analyses show that these filaments may be composed of ferrihydrite; bacteria and their appendages are known to become encrusted with minerals. This may represent the first step in the preservation of the bacterial shapes that leads to the formation of fossils. Alternatively, the forms may be inorganic precipitates. However, they have not been observed in the control basalt sample. In general, the CRB filaments have approximately the same diameter as the proposed nanobacteria observed in ALH84001 (Fig. 3); however, they are usually longer than those observed in the martian meteorite carbonates. Additional work, including analysis for DNA in individual tubular bodies in CRB samples, is presently under way.

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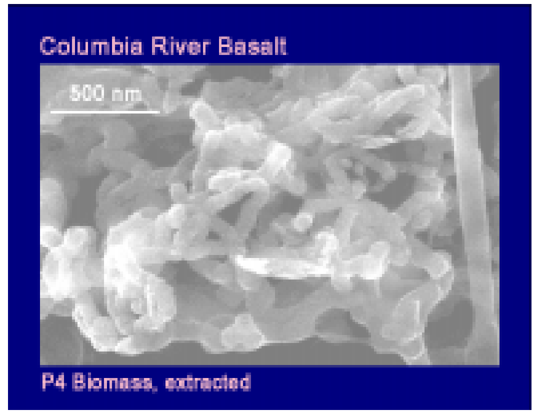

Figure 1. Typical mass of tubular filaments from CRB, P4 biomass sample. This clump of filaments was extracted from a chip surface and was imaged on a cu grid using TEM.

Figure 2. Individual filaments on a CRB chip from the P4 biomass sample. One filament (arrow) is ~350 nm long and 20 nm wide.

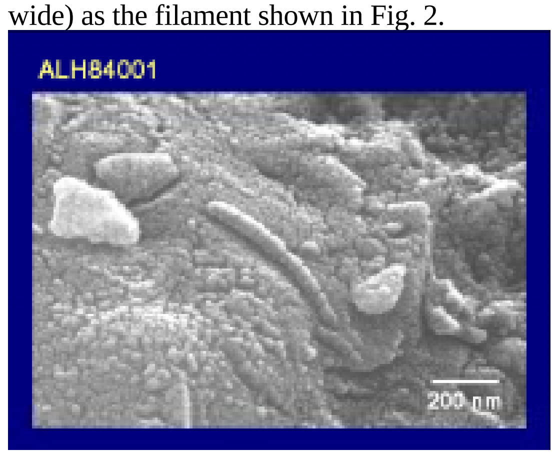

Figure 3. Proposed nanofossil from the martian meteorite ALH84001. This object is approximately the same size (~500 nm long and 30 nm wide) as the filament shown in Fig. 2.